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(54) Title: PUFA SUPPLEMENTS

(57) Abstract

Edible formulations, such as polyunsaturated fatty acids (PUFAs) such as pharmaceutical compositions or nutritional supplements, are disclosed comprising arachidonic acid (ARA). They are adapted to deliver from 150 mg to 1 g per day of ARA and may contain other PUFAs, for example docosahexaenoic acid (DHA). The DHA dosage is from 400 to 600 mg per day, and the ratio of ARA:DHA may be from 1:5 to 5:1. Pharmaceutical compositions comprising ARA and DHA at a ratio of ARA:DHA of 1:1 to 1:2 are also disclosed, as are foodstuffs comprising 0.1 to 5 % ARA. Such formulations can be used to increase ARA levels *in vivo*, for example in pregnant women or for people who have diseases or conditions associated with low ARA levels.

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PUFA SUPPLEMENTS

This invention relates to the provision of polyunsaturated fatty acids (PUFAs) in the diet of humans and animals. More specifically it relates to the provision of polyunsaturated fatty acids of the n-6 and the n-3 families, and in particular the n-6 fatty acid arachidonic acid (ARA) and the n-3 fatty acid docosahexaenoic acid (DHA), and ratios thereof in balanced amounts.

A number of PUFA-containing compositions are currently marketed. EFANATAL™ are capsules, two capsules to be taken per day to give a daily intake of DHA (125mg), ARA (8.6mg) and GLA (40mg). The capsules contain an oil that is primarily based on fish oil. The Applicant has found that this decreases *in vivo* ARA levels, because the DHA content relative to the ARA content in the capsules is too high. Thus this product is in fact an ARA lowering, rather than ARA increasing, composition

despite the fact that it contains ARA. A comparison between this product and those of the invention is provided later.

EFAMARINE™ is also capsules, containing primarily fish and evening primrose oils, of which two are to be taken per day to give a daily intake of EPA (34mg), DHA (22mg) and GLA (68mg).

EPALEX™ is an oil blend, where a teaspoon (5ml) is intended to be taken twice a day, each teaspoon giving DHA (100mg), GLA (21mg), ARA (8mg) and thyme oil (6mg).

Summary of the Invention

A first aspect of the present invention relates to an edible formulation comprising ARA in an amount adapted to deliver a dosage (of ARA) of from 150mg to 1g per day.

Preferably the formulation is adapted to deliver from 200 to 900mg per day ARA, such as from 200 to 700mg per day, optimally from 250 to 400 or 500mg per day.

Edible formulations include dietary supplements and (pharmaceutical) formulations and preparations, such as tablets, pills and capsules. They additionally include (solid or liquid) foodstuffs, for example dairy products (margarine, butter, milk, yoghurt), bread, cakes; drinks such as beverages (tea, coffee, cocoa, chocolate drinks), fruit juices, soft (e.g. fizzy) drinks; confectionery; oily foods (snacks, salad dressing, mayonnaise), soups, sauces, carbohydrate-rich foods (rice, noodles, pasta), fish-containing foods, baby foods (such as infant formula, either as a liquid or powder), pet food, and ready prepared or microwaveable foods.

The ARA can be from any suitable source. It may be from a natural (e.g. vegetable or marine) source, or it may be from a microbial source or from a microorganism, such as fungus, bacterium or a yeast.

Suitable fungi are of the order *Mucorales*, for example *Mortierella*, *Pythium* or *Entomophthora*. The preferred source of ARA is from *Mortierella alpina or Pythium insidiosum*. Suitable commercially available ARA oils include those from DSM/Gist-brocades, Wateringseweg, P.O. Box 1, 2600 MA, Delft, The Netherlands under the trade mark OPTIMAR™ and from Martek Corporation, 6480 Dobbin Road, Columbia, MD 21045, USA, under the trade mark ARASCO™.

In addition to the ARA, one or more additional PUFAs may be provided. This may be another n-6 PUFA in addition to ARA (such as a C18, C20 or C22 fatty acid) or it may be a n-3 fatty acid (for example, a C18, C20 or C22 fatty acid) and in particular EPA and/or DHA. Each PUFA that may be used in the invention may be in the form

of a free fatty acid, fatty acid ester (e.g. methyl or ethyl ester) as a phospholipid or as a triglyceride.

If the formulation comprises an n-3 fatty acid, it is preferred that this is EPA or DHA. If it is DHA, then the formulation is preferably adapted to deliver the same dosage as specified for ARA, such as from 400 to 600mg per day DHA. Alternatively, or in addition, if the formulation comprises EPA, then it is preferably adapted to deliver a dosage of from 150mg to 1g per day EPA, such as from 250 to 500mg of EPA per day.

If the formulation is to be taken (eaten or ingested) once a day then it can contain from 150mg to 1g of ARA. If twice a day then the formulation can have 75mg to 0.5g of ARA, for three times a day a content of 50mg to 330g ARA, and so on, pro rata, for more frequent administrations. The same calculations can be applicable for other PUFAs that may be present, such as DHA.

If the formulation comprises more than one PUFA then the amount of each PUFA can be expressed relatively, as a ratio. For example, if an n-3 PUFA is additionally provided, then the ratio of ARA:n-3 PUFA (such as DHA or EPA) can be from 1:5 to 5:1, preferably from 2:1 to 1:3, optimally from 1:1 to 1:2. The relative amounts of the PUFAs can be balanced so that PUFA levels are supplemented, increased (or at least not decreased significantly) bearing in mind the condition of the individual.

Preferably the PUFA is present in an oil. This may be a pure oil, a processed (e.g. chemically and/or enzymatically treated) or concentrated oil. This oil may comprise from 10 to 100% of the PUFA, but the content may be from 20 to 45%, optimally from 30 to 45% of the desired PUFA, for example ARA, if a microbial oil. Of course, this oil may contain one or more PUFAs within these percentage concentrations. The oil may be a single oil derived from a single cell or a microbial source, or it may be a blend or mixture of two or more oils from these or other (e.g. vegetable or marine) sources. The oil may contain one or more antioxidants (e.g. tocopherol, vitamin E, palmitate) for example at a concentration of from 50 to 800ppm, such as 100 to 700ppm. Suitable processes for preparing PUFAs are described in International patent application numbers PCT/EP97/01446 (WO-A-97/36996), PCT/EP97/01448 (WO-A-97/37032), and PCT/US92/00517 (WO-A-92/13086).

A second aspect of the invention relates to a (pharmaceutical) composition comprising ARA and DHA at a ratio of ARA:DHA of from 1:1 to 1:2. This ratio of PUFAs has been found to provide a good balance, and can increase *in vivo* DHA levels without ARA levels being suppressed due to a too high DHA content. The DHA can be

from a natural (e.g. marine) source or from a microbial source (e.g. from an algae).

A third aspect relates to an edible formulation (eg. a foodstuff) comprising from 0.1 to 3 or 5% ARA. Preferably, the amount is from 0.5 to 1.5 or 2%, optimally from 0.3 to 0.8%. Suitable foodstuffs have already been discussed in relation to the first aspect. Preferred methods of preparing infant formula are disclosed in International application numbers PCT/EP97/01447 (WO-A-97/35487) and PCT/EP97/01449 (WO-A-97/35488).

Suitable formulations can include oils, for example to be taken orally. The oil may be taken as such, or it may be encapsulated, for example in a shell, and may thus be in the form of capsules. The shell or capsules may comprise gelatin and/or glycerol. The formulation may contain other ingredients, for example flavourings (e.g. lemon or lime flavour).

The Applicant has found that certain diseases or conditions, in particular neuronal diseases, are associated with low levels of *in vivo* PUFAs, in particular low levels of ARA in the blood. It is therefore thought that the administration of ARA, or a balance of the PUFAs, will be able to assist in the prophylaxis, prevention, amelioration or treatment of these diseases or conditions. The diseases in question include: neuronal disease, such as schizophrenia,

Parkinsons' disease, osteoporosis, Alzheimer's disease or phenylketonuria.

Examples 1 to 3: Preparation of a composition containing balanced proportions of PUFAs.

This example describes the blending of n-6 and n-3 oils so that they can be included in a single capsule.

The composition was prepared by combining one n-6 PUFA-rich oil with three different n-3 PUFA-rich oils. The n-6 PUFA-rich oil was derived from the fermentation of the filamentous fungus *Mortierella alpina*, and contained approximately 40% ARA as the major fatty acid. For the n-3 PUFA-rich oil the three different sources were: a

high-EPA (above 45%) low-DHA (about 10%) fish oil (from Pronova, Norway under the trade name EPAX™, product no. EPAx4510TG), a high-DHA (above 50%) low-EPA (about 20%) fish oil (also from Pronova under the same brand name, product no. EPAx2050TG), and an oil derived from fermentation of the unicellular alga *Crypthecodinium cohnii* which contains 40% DHA as major fatty acid but is virtually devoid of EPA (from Martek Corporation, Columbia, United States of America under the trade name DHASCO™).

The oils were mixed in appropriate quantities to give the desired amounts and proportions of n-3 and n-6 PUFAs. Here the ARA:DHA ratio for the three blends (Examples 1 to 3) was 1:1. During this procedure, the oxidation-sensitive oils were protected from environmental oxygen by a blanket of oxygen-free nitrogen gas. Subsequently, the oils were used to prepare soft-gel gelatin capsules, where each capsule had 400mg ARA and 400mg DHA.

CLAIMS

1. An edible formulation comprising arachidonic acid (ARA) in an amount adapted to deliver a dosage of from 150mg to 1g/day ARA.

2. A formulation according to claim 1 which is adapted to deliver from 250 to 500 mg/day ARA.

3. A formulation according to claim 1 to 2 which is additionally adapted to deliver docosahexaenoic acid (DHA).

4. A formulation according to any preceding claim which is adapted to deliver a dosage of from 400 to 600 mg/day DHA.

5. A formulation according to any preceding claim wherein the ratio of ARA:DHA is from 1:5 to 5:1, such as from 1:1 to 1:2.

6. An edible formulation comprising from 150 to 700 mg ARA which is intended to be ingested once per day.

7. An edible formulation comprising from 75 to 350 mg ARA which is adapted to be ingested twice per day.

8. An edible formulation according to any preceding claim which is a food or nutritional supplement.

9. An edible formulation according to any preceding claim which is a pharmaceutical composition.

10. A pharmaceutical composition comprising ARA and DHA at a ratio of ARA:DHA at from 1:1 to 1:2.

11. A foodstuff comprising from 0.1 to 5% ARA.

13. The use according to claim 12 wherein the ARA is ingested at from 150 to 700, such as from 250 to 500, mg/day.

14. The use of ARA as a dietary or nutritional supplement for a human who is over 50 years old, preferably over 65 years old.

16. The use of ARA for the manufacture of a medicament for assisting in the prophylaxis, prevention, amelioration or treatment of a disease or condition associated with an abnormal or low level of an n-3 or n-6 PUFA in the blood.

17. The use according to claim 16 wherein the disease or condition is a neuronal disease, such as schizophrenia, Parkinsons' disease, Alzheimer's disease

19. The use of ARA and DHA in an edible formulation at an ARA:DHA ratio that increases the ARA level in blood.

20. The use according to claim 19 wherein the ratio of ARA:DHA is from 1:5 to 5:1.

21. The use according to claim 20 wherein the ratio of ARA:DHA is from 1:1 to 1:2.